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ISOLATION AND IDENTIFICATION OF THE ACTIVE COMPOUNDS IN ESSENTIAL OIL *OCIMUM KILIMANDSCHARICUM* AND THEIR INSECTICIDAL ACTIVITY AGAINST COTTON LEAF WARM

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ABSTRACT

The present study was conducted to extract the essential oils and the crude volatile mixture obtained by steam distillation of *Ocimum kilimandscharicum*, which subjected to GC/MS. The half lethal concentrations (LC50) against *Spodoptera littoralis* . Were (0.073, and 0.072%) for whole essential oil *Ocimum kilimandscharicum* and its active component of plant oil (campher) which isolated from *Ocimum kilimandscharicum*, and identified by using GC/MS.

INTRODUCTION

The leaf oils of the essential oil of *Ocimum sp*. was analyzed by using GC/MS (Maria *et al.,* 2004; Maria *et al.,* 2009). Aromatic and medicinal plants have proved to be an important resource of biologically active compounds useful in medicine and plant protection (Juard and Manners, 1980; Trease and Evans, 1985; Towers *et al.,* 1989; Ashrafuzzaman *et al* 1990; Ansari, 1995. Recently, Wood *et al.,* 1997; Jeyalakshmi and Seetharaman 1998; Radwan 2001;Osman &Radwan 2002; Runyoro *et al.,* 2009; and Maria *et al.,* 2009, investigated the insecticidal and biological effect of plant extracts on the different types of insect larvae.

MATERIALS AND METHODS

(1)Plant materials:

The experimental plant was *Ocimum kilimandscharicum.*

Source: The plant used was collected during 2008 at flowering period.

(2) Experimental Design:

Isolation of tested compounds:

Essential oils: The leaves *Ocimum kilimandscharicum* were collected from medical and aromatic plant research station at El-Kanater–El Qalubyia governorate, Hort. Res. Inst., Agric. Res .Center, Giza, Egypt. The essential oils were separately obtained from the aerial parts of the plants by stem distillation using a Clevenger – type apparatus. Triplicate distillations were performed in succession of 500g for each sample of fresh herbage. Oil of *O. kilimandscharicum* plant from the same growth location were combined and dried over anhydrous Na_2SO_4 and stored at 4°C until used for chemical analysis.

Chemical analysis of volatile oil:

The essential oil was analyzed by GLC Hewlett Packard 5890 gas chromatograph equipped with a FID detector. Samples (1µ) were injected in the split ratio 100:1 in a Supelcowax 10 fused silica capillary column of 50m×0.2mm, 0.33µm.film thickness. The chromatographic conditions were as follows: injector temperature was 250°C; detector temperature was 250°C; oven temperature was programmed from 60 to 230°C and the program rate was 3°C/min.The percentage compositions were computed from GC retention time and computer matching with the MS library provided by the Katto Aromatic Company, Elharanya, Giza Governorate. Five concentrations of essential oil (0.01, 0.025, 0.05, 0.075 and 0.1 ppm) were prepared and tested with drops of SISI-6-emulsifable material in water for diluting the oil.

Biological studies:

A susceptible laboratory strain of *Spodoptera littoralis* was reared in the laboratory away from virus and insecticidal contamination, under room temperature 22°C ±2°C and 55-56 RH.The castor leaves were dipped for 10 s in different extracts or substances which were emulsified by adding drops of Sisi -6 and the control castor leaves were dipped in water contain the same amount of sisi-6.The larvae were allowed to feed on treated castor bean leaves in a Petri dish only for 3 days then transferred to un treated leaves until pupation. Each dish contained 15 larvae and replicated four times. The food was renewed daily and larvae were examined for mortality by

(Ascher and Nemny(1974). All mortality data was corrected for natural mortality using Abbott, formula (1925). All the biological data were statistically analyzed according to Steele and Torrie (1966).

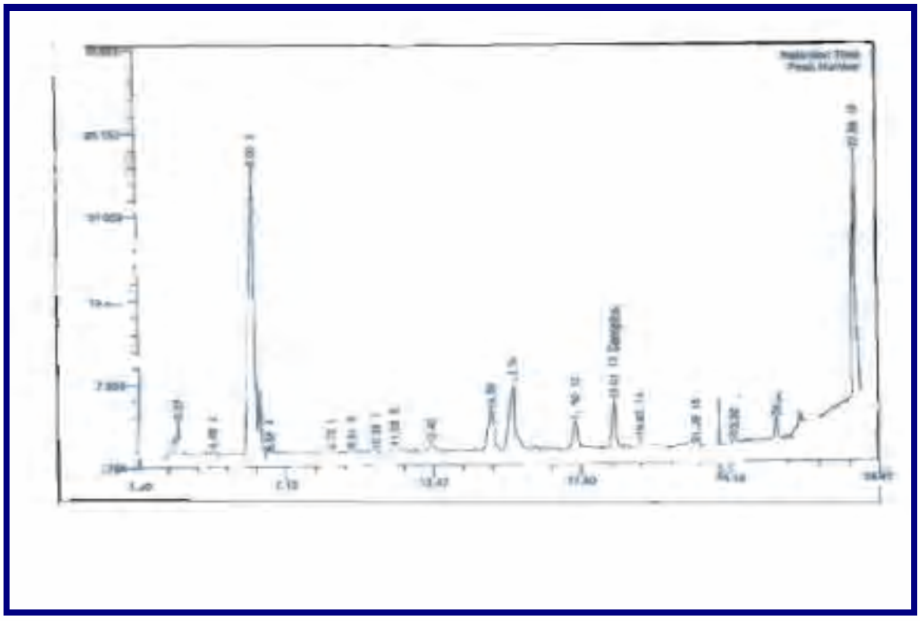
RESULTS AND DISCUSSION

The oil obtained from the stem distillation of the leaves of *Ocimum kilimandscharicum* was 1.98%w/w.The results in (Fig.1) indicated to the retention times of peaks area of composition for the essential oil which were extracted from *Ocimum kilimandscharicum*,. The chromatographic analysis of the oils is presented in (Table1), and the mass spectra of the major peaks are shown in Figure (2). Compositional profiles of *Ocimum kilimandscharicum* essential oil are described in (Table 1). The content of campher which was the main component amounted to (34.39 %).The second major constituent was thymol (20.76 %), and another components detected in significant amounts were ß-caryophyllene (13.40%) , limonine (11.58%) , geraniol (3.98%) , 1-∞-pinene (2.11%) , *p*-Cymin*e* (1.98%) , ß-pinene (1.96%) , menthol (1.16%) ,and myrcene (0.13%).

The GC/MS analysis for the chemical composition of *Ocimum sp.* were found by several investigators (Sekou *et al* .,2000 , Isiaka *et al* .,2003, Brett *et al* .,2004,and Maria *et al* .,2004; Isa *et al*.,2006; Abdullah *et al.;* 2008.;and Maria et al.,2009).

Table (1): Composition of the leaf oils of *Ocimum kilimandscharicum*

Constituents	Percentage composition %
1-∞-pinene	2.11
ß-pinene	1.96
limonine	11.58
campher	34.39
menthol	1.16
thymol	20.76
ß-caryophyllene	13.40
geraniol	3.98
p-Cymin*e*	1.98
myrcene	0.13
Total	**91 .45**



Peak No.	RT.	Peak Width	Area	Height	Area %	Height %	Name of component
1	3.61	4.725	346911	3041	2.11016	5.47474	1-∞-pinene
2	4.27	6.099	323122	951	1.96546	1.71209	ß-pinene
3	6.18	7.747	1903677	6641	11.57951	11.95586	limonene
4	8.18	11.538	123362	485	0. 0.75038	0.87315	unknown
5	12.86	18.242	2367526	6342	14. 40097	11.41756	B-caryophyllene
6	14.55	17.857	3414076	9839	20.76683	17.71325	thymol
7	15.58	10.110	325571	1212	1.98035	2.18198	champher
8	18.79	9.286	265356	928	1.61408	1.67069	menthol
9	20.11	3.352	25982	73	0.15804	0.13142	unknown
10	20.99	8.022	654658	3766	3.98209	6.77997	geraniol
11	22.71	1.099	20551	48	0.12501	0.08641	myrecene
12	23.86	8.791	304627	785	1.85296	1.41234	unknown
13	24.18	14.011	5653930	16662	34.39122	29.99676	carphon
14	25.39	1.484	120168	2375	0.73095	4.27574	unknown
15		3.956	590524	2398	3.59199	4.31714	unknown
Total		**8.4(±)1.065**	**16440041**	**55546**	**100.0000**	**100.0000**	

Fig. (1): Retention times of peaks area and percentage composition for the essential oil, which extracted from *O. kilimandscharicum*.

2.Biological investigation:

Data in Table 2 indicated to the LC_{50} values for essential oil which was extracted from *Ocimum kilimandscharicum* and the active components campher against 4th larvae of *Spodoptera littoralis* .The insecticidal activity of essential oils or aromatic compounds were found by several investigators (Swaran and Dhingra 1996, Behal 1998, and Osman & Radwan 2002). Our results agree with Osman and Radwan (2004), who evaluated the LC_{50} values for *Ocimum kilimandscharicum,* and campher against *S.littoralis* and studied the effect of oils and their active compounds on the biochemical constituents. Abdullah *et al.,*(2008) who studied *Ocimum spp.* and their essential oils have been traditionally used to kill orrepel insects also these study was performed to provide data on the chemical composition with GC/MS.;and Maria *et al.*,2009 studied the bioactivity of three plant drived essential oils against the maize weevils *Sitophilus zeamais* .

Table (2): The LC_{50} values for *O. kilimandscharicum* and Campher against *Spodoptera littoralis.*

Essential oils	*Ocimum kilimandscharicum*	Campher
LC50 (%)	0.073	0.072

1. Active constituents:

Campher was isolated and identified by GC/MS. The chemical structures of Campher are shown in figure (2).

IUPAC name: 1, 7, 7-trimethyl bicycle (2-2-1) heptane-2-one

Formula: C10H16O

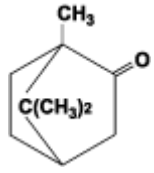


Fig. (2): The chemical structure of campher

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الفصل و التعرف على المكونات الفعالة في الريحان الكافوري و نشاطه الأبادى ضد دودة ورق القطن

الفت عبد اللطيف سيد رضوان

باحث أول بقسم بحوث تحليل المبيدات
المعمل المركزي للمبيدات-مركز البحوث الزراعية-دقي-جيزة

اهتمت هذه الدراسة على فصل الزيت الطيار من نباتي الريحان الكافوري و التعرف على المكون الأكثر فعالية باستخدام جهاز التحليل الكروماتوجرافى الغازي المتصل بجهاز مطياف الكتلة ودراسة تأثيره الأبادى ضد الطور الرابع ليرقات دودة ورق القطن ومعرفة قيمة LC_{50} (التركيز الذي يقتل نصف الحشرات المعاملة) وكانت قيمة LC_{50} 0.073 % لزيت الريحان الكافوري و0.072 % لمركب الكامفر كأحد المكونات الرئيسية في زيت الريحان الكافوري والذي تم التعرف عليه باستخدام جهاز التحليل الكروماتوجرافى الغازي المتصل بمطياف الكتلة.